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A Functional, Cellular, and Evolutionary Model of Nociceptive Plasticity in *Aplysia*

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Abstract. Nociceptive plasticity is defined as behavioral and cellular modification produced by activation of nociceptors. A brief survey of nociceptive plasticity in *Aplysia* reveals a puzzling mixture of behavioral modifications of opposite sign and widely varying durations. These include general sensitization, site-specific sensitization, response-specific facilitation, and inhibition of defensive responses. This behavioral complexity is more than matched by the complexity of cellular correlates reported for the behavioral modifications. A functional model is proposed linking complex patterns of behavioral and neural plasticity in *Aplysia* to potentially general principles of nociceptive function. This model is centered around three overlapping but functionally distinct phases: injury detection, escape, and recuperation. A hypothesis about the early origin of nociceptive plasticity in primitive mechanosensory neurons is then developed, based on similarities in the organization and modifiability of nociceptive systems in evolutionarily divergent groups (primarily mollusks and mammals) and on inferences about the early adaptiveness of postinjury behavioral plasticity. Preliminary evidence suggests that aspects of nociceptive plasticity, and perhaps other forms of memory, may have been derived from cellular repair and signal compensation mechanisms.

Introduction

Neuronal mechanisms controlling withdrawal responses in the opisthobranch mollusc, *Aplysia californica*, have been studied extensively by neurobiologists for over two decades. Investigators have been attracted to the gill, siphon, and tail withdrawal responses of *Aplysia*, in part because the CNS can be readily analyzed in this animal, but largely because these responses and their underlying neurophysiology display a remarkable degree of modifiability. The rare opportunity, provided by *Aplysia* and several other gastropod mollusks, to link behavioral and cellular alterations has been used to advantage by several laboratories and has led to the discovery of various mechanisms contributing to learning and memory in this species.

Although we presume that some mechanisms from gastropod "model systems" are general, the possibility that a given mechanism will be common to groups as evolutionarily divergent as are the mollusks and mammals requires serious scrutiny. As a first step in examining the potential generality of mechanisms contributing to learning and memory in *Aplysia*, I will discuss some nociceptive functions of these mechanisms, propose a three-phase model of nociceptive plasticity, and consider the possibility that mechanisms of nociceptive plasticity evolved in primitive mechanosensory neurons and have been conserved in diverse phyla. Central to this discussion is that most cellular mechanisms of behavioral modification revealed to date in *Aplysia* have been produced either by noxious stimulation, or by manipulations that mimic effects of noxious stimulation. The behavioral and cellular modifications are thus examples of *nociceptive plasticity*, which I define as modifications induced by the activation of nociceptors. Nociceptors are defined as sensory neurons that are activated maximally by stimuli that, if sufficiently prolonged, cause tissue damage (Sherrington, 1906).

Forms of Nociceptive Plasticity

Noxious stimuli, such as strong pinch or shock, were at first assumed to have only two major effects on *Aplysia*:

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Abbreviations: Activity-dependent extrinsic modulation, ADEM; central nervous system, CNS; Excitatory postsynaptic potential, EPSP; Phe-Met-Arg-Phe-NH_2, FMRFamide; serotonin, 5-HT.

to trigger vigorous defensive responses, and to cause general sensitization of the animal for several minutes. The term "sensitization" has been used independently by psychologists and physiologists to describe an increase in sensitivity or magnitude of, respectively, a behavioral or physiological response. I define nociceptive sensitization as sensitization produced by noxious stimulation, where sensitization is defined physiologically as an increase in sensitivity or responsiveness of the organism to a constant test stimulus. Such hypersensitivity after noxious stimulation need not be expressed as overt behavior, but is often expressed as a decrease in threshold and an increase in the magnitude of defensive responses evoked by a test stimulus. By this definition, sensitization may also be expressed as inhibition of ongoing behavior (usually nondefensive) by the test stimulus. Nociceptive sensitization can be general (expressed by changes in response to a broad range of test stimuli and stimulation sites) or, as discussed below, specific to a warning signal or to a restricted site on the body. The apparent function of general nociceptive sensitization is to prime the animal for continued defense, so that it responds rapidly and energetically to a wide range of stimuli that might presage an attack.

The first clue that nociceptive plasticity involves more than a brief, general sensitization came from the observation that repeated application of noxious stimuli over hours or days causes general sensitization of siphon withdrawal that can last for weeks (Pinsker *et al.*, 1973; Frost *et al.*, 1985). It was then discovered that sensitization can be conditioned to a warning signal; a variety of defensive responses are selectively facilitated by a chemosensory cue (*e.g.*, shrimp extract) if it is repeatedly paired with noxious shock (Walters *et al.*, 1981; Colwill *et al.*, 1988). Further links between sensitization and associative processes were indicated by behavioral data (Carew *et al.*, 1981, 1983; Hawkins *et al.*, 1983) and neuronal data (see next section), suggesting considerable overlap of sensitization and putative classical conditioning mechanisms within individual sensory neurons.

The next discovery, site-specific sensitization, is crucial to the functional and evolutionary arguments of this paper. Noxious stimulation enhances siphon and tail withdrawal test responses; but responses evoked by test stimuli applied near the site of noxious stimulation are more dramatic than those evoked by test stimuli applied at other sites on the body (Walters, 1987a). The site-specific behavioral plasticity is particularly potent; a single 45 s noxious stimulation sequence that is insufficient to cause long-term general sensitization produces site-specific sensitization lasting a week or more (Fig. 1).

The complexity of nociceptive plasticity was underscored recently when several groups found that noxious stimulation can inhibit, as well as enhance, defensive responses (Krontiris-Litowitz *et al.*, 1987; Mackey *et al.*, 1987; Marcus *et al.*, 1988). The most complete behavioral study of nociceptive inhibition was reported by Marcus *et al.* (1988), who showed that inhibition of siphon withdrawal occurs following noxious but not innocuous stimuli, and that net inhibition has a brief duration.

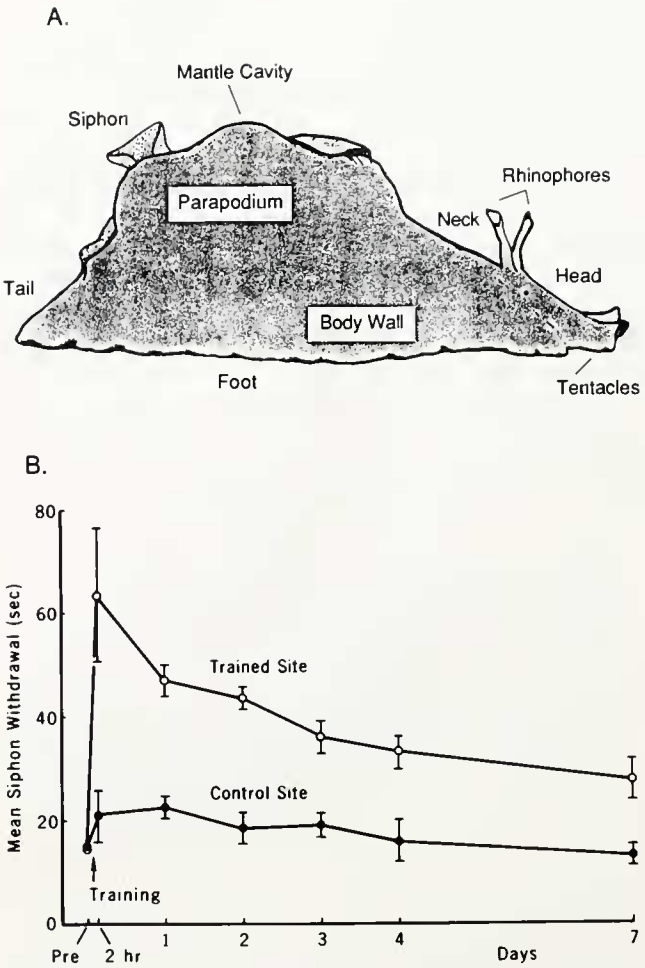

Figure 1. Site-specific sensitization following noxious tail stimulation in freely moving *Aplysia*. (A) Diagram of unrestrained animal used for testing and training. Before and after site-specific sensitization training, weak test stimuli were applied with a hand-held electrode to a site on each side of the tail that had been marked with a suture (not shown). Training consisted of a 45 s sequence of strong shocks to one of the test sites. During each test, the duration of siphon withdrawal was timed, and the magnitude of tail withdrawal was estimated. (B) Site-specific sensitization of siphon withdrawal. Siphon withdrawal was significantly greater when tested at the trained site than the contralateral control site. Similar differences were seen in tail withdrawal (not shown) and when other parts of the body were trained and tested (Walters, 1987a).

These various forms of nociceptive plasticity differ in the sign, duration, and stimulus specificity of behavioral modulation. Yet another dimension of plasticity was revealed by the discovery that particular siphon responses are modulated selectively by noxious stimulation of dif-

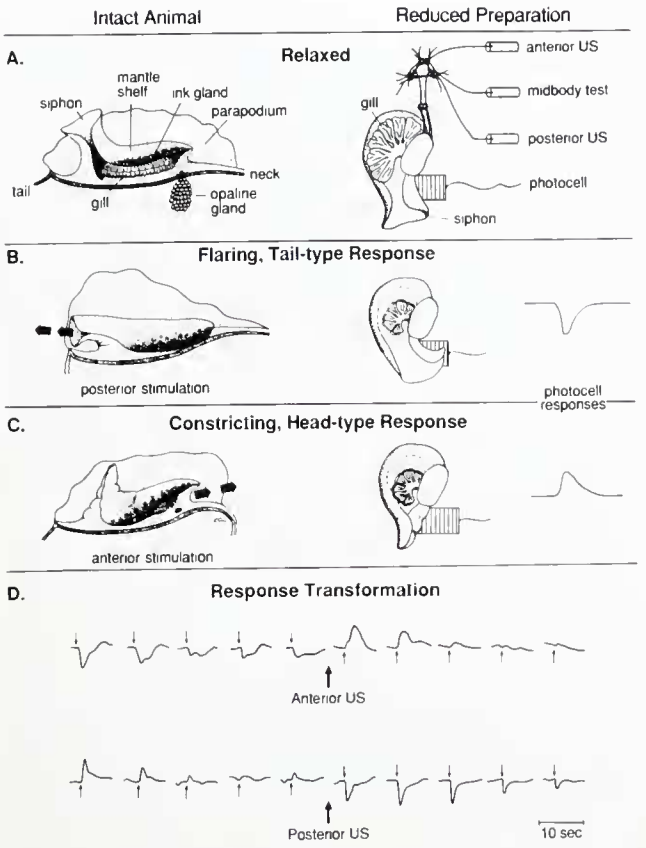

Figure 2. Transformation of siphon responses following noxious stimulation. The left column shows a cutaway view of the siphon and mantle organs in the intact animal (compare Fig. 1A). The right column shows the mantle organs and CNS in a reduced preparation. The photocell monitors the breadth but not the length of the siphon. (A) Relaxed siphon. Weak test stimuli were applied to a midbody nerve at 1 min intervals. A noxious unconditioned stimulus (US), a 15 s sequence of strong shock, was delivered to either a tentacle nerve or a tail nerve. (B) Flaring response, typical of posterior stimulation. The photocell shows a negative deflection. (C) Constricting response typical of anterior stimulation. The photocell shows a positive deflection. (D) Examples of transformed responses. Top—flaring responses are converted to constricting responses after noxious anterior stimulation. Bottom—constricting responses are converted to flaring responses after noxious posterior stimulation (Erickson and Walters, 1988).

ferent regions of the body. This response-specific nociceptive plasticity is expressed most clearly when noxious stimulation causes the animal to respond to a test stimulus with a qualitatively different response than it did before noxious stimulation (Erickson and Walters, 1988). Figure 2 shows examples of siphon responses being transformed into opposite responses following intense stimulation of nerves from the head or tail. Like sensitization, response transformation can be enhanced by associative training. The incidence and degree of transformation of motor responses to particular test stimuli are preferentially increased if the test stimulus is repeatedly paired with a noxious stimulus (Walters, 1989; Hawkins *et al.*, 1989).

Mechanisms of Nociceptive Plasticity

Mechanisms of general sensitization in *Aplysia* have received detailed analysis. Here I briefly describe selected aspects of cellular mechanisms, focusing on those that have been closely linked to changes in defensive behavior. For reviews of subcellular mechanisms of sensitization, see Kandel and Schwartz (1982) and Byrne (1987).

In principle, sensitization might involve alterations in any of various classes of neurons known to contribute to defensive behavior in *Aplysia* (Fig. 3). Although some interneurons and motor neurons show alterations during general sensitization (*e.g.*, Frost *et al.*, 1988), analysis has centered on mechanosensory neurons: the LE cluster, which innervates the siphon (Byrne *et al.*, 1974); and the VC clusters, which innervate most of the rest of the body (Walters *et al.*, 1983a). No major differences between these sensory clusters have been described in their response properties or plasticity. Cells in both clusters show a wide dynamic range, responding weakly to stimuli of moderate intensity and more strongly as stimulus intensity is in-

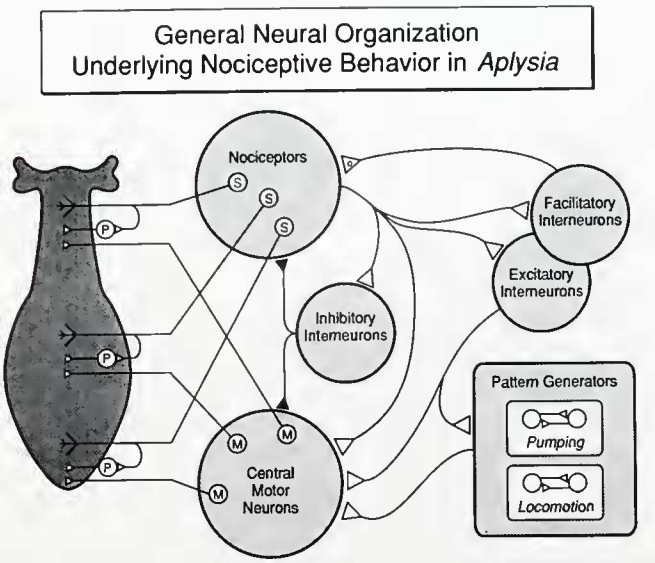

Figure 3. General pattern of neural organization controlling nociceptive behavior in *Aplysia*. Each indicated population of cells may include hundreds of neurons distributed throughout the nervous system. Wide-dynamic range nociceptive sensory neurons (S) innervate the entire body surface. Each cell connects to peripheral motor neurons (P), to central motor neurons (M) innervating the same region, and to inhibitory, excitatory, and facilitatory interneurons. Sensory neurons also make connections (largely polysynaptic) to complex pattern generating networks responsible for rhythmic defensive behaviors such as mantle pumping (used to eject ink and to increase respiration and blood circulation) and escape locomotion. Relatively little is known about interconnections among the various types of interneurons. A further complication is that some interneurons are multifunctional (*e.g.*, having both excitatory and facilitatory effects on the same follower neuron). Based on data from Bailey *et al.* (1979), Byrne (1980, 1983), Hawkins *et al.* (1981), Frost *et al.* (1988), and Hickie and Walters (unpub. obs.).

creased (Byrne *et al.*, 1978; Walters *et al.*, 1983a). Both clusters have nociceptive functions, because they respond maximally to noxious pinching stimuli (Walters *et al.*, 1983a; Walters and Clatworthy, unpub. obs.), and they are therefore indicated, in Figure 3, within the circle labeled "nociceptors".

Sensory neurons in the LE cluster (Bailey *et al.*, 1979), and probably in other central nociceptive clusters (*e.g.*, Walters, 1987b), make some synaptic connections to peripheral motor neurons (Fig. 3). But with few exceptions (see Clark and Kandel, 1984), analysis of synaptic plasticity in these sensory populations has focused on their strong monosynaptic connections to identified motor neurons within the CNS. Because of these connections and others to excitatory, facilitatory, and inhibitory interneurons involved in defensive responses (Fig. 3), changes in the signalling properties of LE and VC sensory neurons should have potent effects on behavioral responses elicited by moderate to strong cutaneous stimuli. Short-term behavioral sensitization is correlated with general facilitation of synapses from sensory neurons to motor and interneurons (Kandel and Schwartz, 1982; Walters *et al.*, 1983b) and with increased excitability of peripheral branches of the sensory neuron (Clatworthy and Walters, 1990). The presynaptic facilitation is mediated, at least in part, by 5-HT (Glanzman *et al.*, 1989), which can also enhance excitability of the central and peripheral parts of the sensory neuron (Walters *et al.*, 1983b; Klein *et al.*, 1986; Billy and Walters, 1989b). Many of the effects of 5-HT are mediated by cyclic AMP-dependent protein kinase (Kandel and Schwartz, 1982), and some are mediated by protein kinase C (Braha *et al.*, 1990). The most notable effects involve the depression of K^+ conductances (Klein *et al.*, 1982; Baxter and Byrne, 1989; Walsh and Byrne, 1989), which increase transmitter release and excitability by broadening spikes and decreasing spike accomodation (Kandel and Schwartz, 1982; Walters *et al.*, 1983b; Klein *et al.*, 1986). Noxious stimulation also appears to enhance a Ca^{2+} conductance (Edmonds *et al.*, 1990).

The expression of long-term sensitization in sensory neurons involves some of the same mechanisms as short-term sensitization: depressed K^+ conductances, increased transmitter release, and increased excitability (Frost *et al.*, 1985; Scholz and Byrne, 1987; Walters, 1987b). Specific morphological changes also occur in the sensory neuron, including the growth of new synaptic varicosities and active zones within the CNS (Bailey and Chen, 1983, 1988; Nazif *et al.*, 1989), and possibly the growth of peripheral processes that expand the size of the receptive field (Billy and Walters, 1989a). Considerable effort is being made to identify molecular mechanisms involved in inducing and maintaining long-term changes in these sensory neurons (*e.g.*, Barzilai *et al.*, 1989; Eskin *et al.*, 1989).

Associative enhancement of withdrawal responses to mechanosensory cues and site-specific sensitization appears to involve the same basic mechanism: activity-dependent enhancement of the mechanisms of general sensitization, as described above (Walters and Byrne, 1983a; Hawkins *et al.*, 1983; Walters, 1987b). Figure 4 illustrates two of the sensory neuron alterations contributing to long-term site-specific sensitization: synaptic facilitation and increased soma excitability; the latter is expressed dramatically as a prolonged afterdischarge to brief depolarization. During the induction of site-specific sensitization, the sensory neuron is activated by the noxious stimulus. In associative conditioning, the sensory neuron is activated by a cue presented immediately before the noxious stimulus. In both cases, the activity enhances the effects of extrinsic chemical modulators (*e.g.*, 5-HT) on the sensory neuron, and thus this general class of plasticity is termed activity-dependent extrinsic modulation (ADEM). Activation of sensory neurons opens Ca^{2+} channels (Walters and Byrne, 1983b; Edmonds *et al.*, 1990). The resulting Ca^+ influx enhances adenylate cyclase activity, increasing the rate of cyclic AMP synthesis, and thus amplifying the degree and duration of plasticity induced by neuromodulators released during noxious stimulation (Abrams and Kandel, 1988). Ca^{2+} might also enhance plasticity in other ways; for example, Ca^{2+}-dependent kinases may directly phosphorylate transcription factors (*cf.* Dash *et al.*, 1990).

The cellular mechanisms of nociceptive inhibition have also been studied. Mackey *et al.* (1987) reported that tail shock causes presynaptic inhibition and spike narrowing in siphon sensory neurons, and that these effects are partly mediated by an identified interneuron containing the neuropeptide FMRFamide. Application of FMRFamide to the sensory cell soma and synaptic region in the CNS causes hyperpolarization, spike narrowing, and presyn-

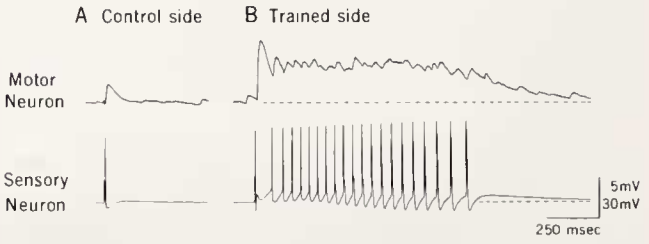

Figure 4. Example of synaptic facilitation and afterdischarge in a sensory neuron after site-specific sensitization. The intact animal was trained with strong noxious shock delivered to one side of the body. One day later, the CNS was removed and sensory and motor neurons innervating the trained side and corresponding regions on the contralateral side were examined. (A) Typical connection between a tail sensory neuron and tail motor neuron innervating the untrained side of the tail. The connection was tested by activating the sensory neuron with a 10 ms depolarizing pulse injected into the soma. (B) The same test procedure on the trained side elicits a larger synaptic potential and an afterdischarge of 20 spikes (Walters, 1987b).

Table I

Three-phase model of the functions and general mechanisms of nociceptive plasticity in Aplysia

	Phase 1. Injury detection	Phase 2. Escape	Phase 3. Recuperation
Period:	0.1 s–10 min	1 s–30 min	10 min–1 month
Functions:	• Severity appraisal • Localization • Compensation for destruction of nociceptive channels • Defensive response triggering • Anticipation	• Flight • Inhibition of competing responses	• General inactivity (healing) • Defensive readiness (sensitization) a. General b. Wound specific c. Cue specific
Mechanisms:	• Nociceptor activation a. Frequency code b. Wide dynamic range • Activation of defensive circuits (Somatotopic organization) • Nociceptor facilitation a. Afterdischarge b. PTP c. HSF d. Hyperexcitability e. ADEM • Motor facilitation	• Activity in circuits generating escape behavior • Nociceptor inhibition a. Presynaptic inhibition (neuromodulation) b. Activity-dependent reduction in excitability • Inhibition of motor and interneurons controlling other behaviors	• Inhibition of circuits controlling feeding, reproduction, etc. • Nociceptor facilitation a. ADEM b. Axon injury signals c. Lower threshold d. Less accomodation e. Afterdischarge f. Synaptic facilitation g. Sprouting • Motor facilitation

The times for each phase indicate the approximate beginning and end of the phase relative to the beginning of the noxious stimulus. ADEM—activity-dependent extrinsic modulation; HSF—heterosynaptic facilitation; PTP—posttetanic potentiation.

aptic inhibition in sensory neurons (Belardetti *et al.*, 1987), whereas peripheral application increases mechanosensory threshold (Billy and Walters, 1989b). Extrapolation from studies in other mollusks suggests that other neuromodulators may also contribute to nociceptive inhibition of defensive responses in *Aplysia*. For example, pharmacological evidence suggests that inhibition of a defensive response in another gastropod, the snail *Cepaea*, may involve opiate-like modulators (Kavaliers, 1987). In *Aplysia*, noxious stimulation also produces activity-dependent reduction in the excitability of sensory neuron axons and receptive fields (Clatworthy and Walters, 1990), and can sometimes block afferent spikes (Clatworthy and Walters, 1989). The presynaptic inhibition produced by FMRFamide is also activity-dependent (Small *et al.*, 1989). Recently, Wright *et al.* (1989) suggested that interneurons may be more important loci than sensory neurons for inhibition in the siphon withdrawal system. They found that tail shock suppressed polysynaptic (interneuronal) components of a complex test EPSP in siphon motor neurons under conditions in which no inhibition of the monosynaptic EPSP from the sensory neurons was detected.

Mechanisms of response-specific nociceptive plasticity are not yet known. However, tests of several potential mechanisms have recently begun in identified neurons within siphon control circuits (Erickson and Walters, 1988; Frost *et al.*, 1988; Hickie and Walters, 1990; Fang and Clark, 1990).

A Functional Model of Nociceptive Plasticity

These findings show that noxious stimulation causes highly complex behavioral and neuronal alterations in *Aplysia*. Two issues have not been clear: the functional significance of this complexity, and the integration of apparently opposing forms of plasticity to produce adaptive behavior. General similarities between the patterns of nociceptive behavior observed in *Aplysia* and in other species (primarily rats and humans) suggest that forms of nociceptive plasticity in *Aplysia* might represent common behavioral adaptations to ubiquitous selection pressures; namely, escape from a source of bodily injury, and optimization of recuperation. This possibility encouraged the formulation of a model linking potentially general principles of nociceptive function to patterns of behavioral and neural plasticity that have been described in *Aplysia*. In a functional model of pain and fear in mammals, Bolles and Fanselow (1980) divided nociceptive responses into perceptual, defensive, and recuperative phases. Somewhat similar phases can be used in a functional model to explain much of the complexity of behavioral and neuronal plasticity observed in *Aplysia* following noxious stimulation (Table I). These overlapping phases of injury detection,

escape, and recuperation correspond to periods of immediate facilitation, short-term inhibition, and long-term facilitation of defensive responses.

Phase 1—injury detection

How does an animal know it is injured, or about to be injured? False negative answers mean an animal will fail to initiate escape and recuperative behavior, jeopardizing its life. False positive answers also reduce biological fitness by committing the animal unnecessarily to energy-consuming escape behavior, and possibly to a long period of recuperative behavior during which important activities such as reproduction and feeding are inhibited. One way for a CNS to decide whether an injury has occurred is to interpret any activity on nociceptive labeled lines from the body as proof of injury. However, because an animal needs to match its responses to the severity and location of its injuries, nociceptive signals should also carry intensity and spatial information. In addition, if the severity of an injury is represented by the number of active nociceptive fibers and by their degree of activity, there should be some way of compensating for the loss of signal strength during and after injury severe enough to destroy or damage nociceptive fibers from the injured region. Finally, a nociceptive system would be highly adaptive if it could recognize noxious stimuli prior to actual injury.

These general functional considerations are reflected in the organization of the nociceptive system of *Aplysia* and in the alterations of this system that immediately follow moderate intensity or noxious cutaneous stimulation (Table I). LE and VC sensory neurons trigger defensive withdrawal responses, and the magnitude of the responses thus evoked depends upon the number of LE or VC neurons activated, upon the number and frequency of spikes generated, and upon the amount of transmitter released per spike (Byrne *et al.*, 1978; Walters *et al.*, 1983a; Walters, unpub. obs.). Therefore, the likelihood and severity of body wall injury in *Aplysia* appear to be coded, at least in part, by the total level of activity in these nociceptive channels and by the strength of nociceptive connections to interneurons and motor neurons. As in mammals, the relatively small size of nociceptive receptive fields and the somatotopic organization of sensory and motor pathways in *Aplysia* (Walters *et al.*, 1983a) contribute to the localization of noxious stimuli.

How does this system compensate for the destruction of nociceptive axons during severe injury? The VC and LE sensory neurons provide labeled lines to the CNS from the periphery, but their wide dynamic range and response properties also make possible a frequency code for the severity of injury. A brief, punctate, moderately intense stimulus to the tail, which does not cause injury unless greatly prolonged, typically evokes one to five spikes in each of three to five VC neurons, the receptive fields of which are estimated to overlap any given point on the tail (Walters *et al.*, 1983a; Billy and Walters, 1989a). The total activity in these sensory neurons (10–20 spikes over about 0.5 s) leads to a relatively brief withdrawal of the tail and siphon, and perhaps to escape locomotion. A strong, punctate, pinching stimulus of the same duration, which may cause some cutaneous damage but does not destroy major axons of VC sensory neurons, causes high frequency activation of each sensory neuron, but the activation rarely outlasts a moderately noxious stimulus. Thus, a 0.5 s stimulus might lead to a 0.5 s barrage of perhaps 75 spikes across the same 5 VC neurons, leading to strong, long-lasting withdrawal of the tail and siphon, as well as to inking and vigorous escape locomotion. Finally, a severe, crushing stimulus of the same duration will probably destroy some of the sensory axons innervating the region. Assuming, for illustrative purposes, that axons from three of the five sensory neurons are destroyed, how is the CNS informed of the severity of the injury? First, the remaining fibers will fire at maximal frequency (about 50 Hz) during the stimulus. Second, the crushed axons will produce an injury discharge of high frequency spikes. Third, very strong stimuli produce an afterdischarge in VC sensory neurons (see Fig. 4) that can last 0.1 to 3 s (Clatworthy and Walters, 1988, and unpub. obs.). The afterdischarge is generated, at least in part, within the CNS (Clatworthy and Walters, 1988), raising the possibility that both the intact VC neurons, and the VC neurons with injury-destroyed axons, fire at high frequency during the noxious stimulus and for 1 to 2 s afterwards. Thus, even with a majority of the sensory fibers from the injured region disconnected from the CNS by the injury, a barrage of 100–200 high frequency sensory spikes may reach central synaptic terminals onto defensive motor and interneurons. The mechanism of afterdischarge is not yet known, but it appears to depend upon both the initial spike activity and the extracellular release of chemical modulators, *i.e.*, ADEM (Clatworthy and Walters, unpub. obs.). Finally, under natural conditions, more severe stimuli usually affect larger areas of body wall and thus activate more nociceptors.

How does the system anticipate injury during moderate intensity cutaneous stimulation that threatens but does not immediately produce tissue damage? Stimuli sufficiently intense to activate LE and VC neurons, but not severe enough to cause immediate body wall injury, have transient facilitatory effects upon defensive responses. This facilitation involves brief (seconds to minutes) heterosynaptic facilitation (Carew *et al.*, 1971; Walters *et al.*, 1983b), post-tetanic potentiation (Walters and Byrne, 1984; Clark and Kandel, 1984), and enhanced peripheral excitability (Clatworthy and Walters, 1990) in the sensory neurons. The facilitation of peripheral excitability, as well

as facilitation of synaptic transmission (Hawkins *et al.*, 1983; Walters and Byrne, 1983a), is greatest in sensory neurons activated by the noxious stimulus. As a consequence, continued or repeated application of a moderately noxious stimulus to the same region should cause increasing activation of the nociceptors, increasing synaptic facilitation (Walters *et al.*, 1983b), and increasingly effective sensory input to the CNS. Temporal summation of excitatory and facilitatory inputs to defensive interneurons and motor neurons occurs (Carew and Kandel, 1977; Walters, unpub. obs.), facilitating motor responsiveness. These effects also increase the spontaneous firing rates of some motor neurons, which can lead to neuromuscular facilitation (*e.g.*, Frost *et al.*, 1988). All of these sensory and motor facilitation mechanisms produce "windup" of neural responses to repeated or prolonged stimulation that is intense enough to be threatening. Windup has two consequences: withdrawal and escape responses are triggered before a prolonged, moderately noxious stimulus injures the animal; and the animal is prepared to respond maximally if more severe stimulation follows. Windup of responses to noxious stimuli in mammals appears to involve some of the same mechanisms (Woolf and Walters, 1991). Brief habituating and inhibitory effects, reported for relatively weak cutaneous stimuli in *Aplysia* (*e.g.*, Kupfermann *et al.*, 1970; Mackey *et al.*, 1987), should oppose and delay these facilitatory effects, reducing the chances of overreaction to innocuous stimuli.

Phase 2—escape

When the CNS interprets a stimulus as injurious or potentially injurious, escape behavior is initiated, and the second phase of nociceptive plasticity begins. It has long been observed that animals in the act of fleeing or fighting ignore their injuries. Nociceptive responses are inhibited, and this inhibition prevents less urgent behavior patterns from interfering with emergency responses critical for escaping from, or repelling, mortal threats (Wall, 1979; Bolles and Fanselow, 1980). In *Aplysia*, strong shock or pinching stimuli inhibit withdrawal reflexes and associated neural activity in an intensity-dependent manner, and the inhibitory effects generally last for 1 to 15 min (Marcus *et al.*, 1988; Walters, Erickson, and Clatworthy, unpub. obs.). This time course and intensity dependence roughly parallel those of escape locomotion (Walters and Erickson, 1986). Because massive withdrawal of any region of the body interferes with escape locomotion, a major function of nociceptive inhibition in this animal is probably to prevent the disruption of escape behavior that would occur if strong withdrawal responses were triggered during flight. As described above, inhibition of defensive responses appears to involve neuromodulation of sensory neurons, interneurons, and perhaps motor neurons, with some of the inhibition being activity-dependent (Table I).

Phase 3—recuperation

After several minutes of escape locomotion, *Aplysia* stop (in a crevice if available), contract into a tight spherical shape, and remain motionless. If the injury is severe, an animal may show little sign of activity for up to several days. If the animal is touched during this time, it will show exaggerated withdrawal responses and a low threshold for escape locomotion, especially if contact is made near the wound (Walters, 1987a, and unpub. obs.). Inactivity during wound healing presumably involves inhibition of circuits controlling active behaviors, such as feeding and mating, which are not immediately essential and which would subject the wound to further stress. Inhibitory signals may include neuroendocrine substances and factors released into the blood from ruptured cells at the site of trauma (Krontiris-Litowitz *et al.*, 1989).

While little is known about mechanisms underlying inhibition of nonessential behaviors during the recuperative phase, a great deal has been learned about the enhancement of defensive responses during this phase. The various mechanisms of sensitization reviewed earlier in this article serve to increase the animal's readiness for defensive action while the wound heals (Table I). This sensitization is functionally equivalent to long-term hyperalgesia in mammals. Hypersensitivity is especially important around the region of injury because a wound may leak substances that can invite further attack from predators or parasites, and because a wounded region is likely to be weakened and vulnerable to further disturbance. Persistent general sensitization in *Aplysia* is mediated, at least in part, by long-term heterosynaptic facilitation of wide-dynamic range nociceptors (Frost *et al.*, 1985; Walters, 1987b). Wound-specific sensitization involves at least three basic mechanisms. First, long-term site-specific sensitization is produced by ADEM of nociceptors activated during wounding. This selectively decreases peripheral mechanosensory threshold (Billy and Walters, 1989a), enhances nociceptor afterdischarge, and produces synaptic facilitation in sensory neurons innervating the wounded region (Walters, 1987b). Second, signals generated at a site of axonal injury may be carried by retrograde axonal transport to the soma and synapses, where they induce the same set of hyperexcitability and facilitatory effects as are triggered by ADEM (Walters, Alizadeh, and Castro, unpub. obs.). The generation of signals at sites of axonal injury may involve interactions with extracellular factors associated with immunocytes aggregating at damaged tissue (Alizadeh *et al.*, 1990). In each case, persistent sensitization may involve growth of new synapses and sprouting of new branches from central and peripheral sensory arbors (Bailey and Chen, 1988; Billy and Walters, 1989a). A third basic mechanism of long-term wound-specific sensitization has been implicated by behavioral

experiments (Fig. 2), but has not yet been demonstrated within the nervous system—selective enhancement of the responsiveness of elements within motor control circuits controlling specific defensive responses appropriate for the wounded region (motor facilitation; Erickson and Walters, 1988).

An interesting feature of nociceptive systems in *Aplysia* and in mammals is the prominence of wide-dynamic range neurons. Results from *Aplysia* suggest that this feature may be important for the induction of nociceptive behavior by innocuous stimuli during nociceptive sensitization (an effect functionally equivalent to allodynia in humans—pain evoked by innocuous stimuli). In *Aplysia* the severity of an injury is partially encoded by the total output of nociceptors representing the injured region (*i.e.*, the number of cells activated × firing rate per cell × transmitter release per spike). Thus, a moderately intense stimulus that would normally be innocuous will be interpreted by the CNS as noxious if transmitter release or spike frequency are enhanced in wide-dynamic range nociceptors after an injury. Presumably, innocuous tactile stimulation near a serious wound is often sufficiently threatening to evoke nociceptive behavior during recuperation in both *Aplysia* and mammals.

The ADEM mechanism in LE and VC sensory neurons may contribute, not only to site-specific sensitization around a wound, but also to classical conditioning of a cutaneous warning cue distant from a wound, provided that the warning cue is at least moderately intense and is delivered shortly before wounding (Walters and Byrne, 1983; Hawkins *et al.*, 1983). However, this cue-specific sensitization mechanism would only be useful if the cue were subsequently to contact the same receptive field. Given the small size of these cells' receptive fields (decreasing the chances of repeated contact), conditioned enhancement of their signals would seem to provide an undependable warning cue (see Walters, 1987b). Cue-specific sensitization mechanisms should be more effective in sensory neurons that have global receptive fields and that can detect a threat at a distance (before contact), allowing more time for avoidance of a threatening situation. Chemosensory neurons have these properties, and chemical stimuli may thus be more effective cues for aversive conditioning than tactile cues. Aversive conditioning with chemosensory cues occurs readily in *Aplysia* (Walters *et al.*, 1981; Colwill *et al.*, 1988), but whether such conditioning is more rapid or potent than conditioning with tactile cues is not yet known (*e.g.*, Carew *et al.*, 1981).

A Hypothesis About the Evolution of Nociceptive Plasticity

Although nociceptive neurons and nociceptive responses have been examined in a variety of species (*e.g.*, Nicholls and Baylor, 1968; Kavaliers, 1988), investigations of behavioral and neuronal *plasticity* following noxious stimulation have largely been restricted to mammalian and molluscan preparations. Nociceptive plasticity in these two groups shows a number of interesting similarities (reviewed by Walters, 1987a,b; Kavaliers, 1988; Woolf and Walters, 1991). In both groups, facilitatory and inhibitory alterations occur in the first stages of nociceptive processing—within wide dynamic range nociceptors in *Aplysia*, and in primary nociceptors and secondary wide-dynamic range spinal interneurons in mammals. Similarities include: intensity-dependent enhancement and inhibition of central excitability, enhanced peripheral sensitivity, enlargement of nociceptive receptive fields, and activity-dependent plasticity. Furthermore, preliminary evidence suggests that aspects of the underlying subcellular mechanisms (*e.g.*, depressed K^+ conductances, mediation by common protein kinases, and activation of "immediate-early" genes) might also be shared (see Woolf and Walters, 1991). In principle, the similarity of any given feature may be due to either convergent evolution of independent mechanisms in response to common environmental pressures (analogy), or to conservation of primitive mechanisms that had evolved in ancestors common to both mollusks and mammals (homology). The ancestors of mollusks and mammals diverged very early in the history of the animal kingdom, before the protostome and deuterostome lineages split during the Precambrian era. Thus, homologous features in mollusks and mammals must be very primitive, having descended from small, soft-bodied animals that lived more than 600 million years ago, before the hard shells or skeletons that would leave a fossil record had evolved (Avers, 1989).

To what extent are mechanisms of nociceptive plasticity homologous in mollusks and mammals? Undoubtedly many similarities are due to analogous adaptations developed independently by these groups in response to a ubiquitous pressure—the dangers that follow sublethal injury in a hostile environment. On the other hand, two arguments suggest that this same pressure existed during the evolution of primitive common ancestors of mollusks and mammals, and could have supported the early evolution of adaptations to optimize defensive behavior following noxious stimulation. First, the presence of predators during early periods of animal evolution is suggested by the occurrence of withdrawal and escape responses in virtually all existing animal groups, including protozoans (Kavaliers, 1988). Unfortunately, almost nothing is known about predators in the Precambrian world except that they, like their prey, would have been small and soft-bodied, and probably lacked specialized feeding appendages (Vermeij, 1987). Nevertheless, Precambrian predators probably existed (Hickman *et al.*, 1984) and would not have needed specialized appendages. For example, prey

may have been captured with nets of mucus, and eaten with simple grasping and extracellular digestion methods, similar to those used by some flatworms today. Second, injury could have also been produced by random assaults from the environment, such as wave action. An injured animal would have been more vulnerable to further physical disturbance, and to detection and attack by predators.

If early mechanisms of nociceptive plasticity did, in fact, originate in primitive animals having very simple nervous systems, an attractive possibility is that some of these mechanisms first appeared in primary mechanosensory neurons. These cells were among the earliest neurons (*e.g.*, Bullock and Horridge, 1965). Because they are directly exposed to surface trauma, they provide a single locus for both recognizing noxious stimulation and altering the responses of an animal to subsequent mechanical stimulation. Exposure of the peripheral branches of mechanosensory neurons to surface trauma suggests a specific cellular hypothesis for the origin of some mechanisms of nociceptive plasticity. Trauma to the body surface (or even wear on soft body parts in a turbulent environment) can damage peripheral sensory branches. The ubiquity of cellular repair processes in modern cells suggests that such processes appeared very early in the evolution of life and were available to repair damaged sensory branches in primitive animals. For an organism to take mechanisms that had evolved to regenerate and maintain excitability in damaged neuronal branches, and use these mechanisms in undamaged neurons where they could amplify the neurons' normal signalling effectiveness, seems a small step. For example, mechanisms of cellular repair, growth, and signal compensation triggered by intracellular signals of cellular injury might become inducible by extracellular neuromodulators released during noxious stimulation, or by interactions of such neuromodulators with spike activity in the neuron (*i.e.*, ADEM).

Recently we tested whether a close relationship exists between mechanisms of general and site-specific sensitization on the one hand (*i.e.*, involving neuromodulation and ADEM), and responses to axonal injury on the other (Walters, Alizadeh, and Castro, unpub. obs.). We studied the effects of axonal injury by crushing nerves containing sensory neuron axons under conditions in which synaptic release of neuromodulators (such as 5-HT) and ADEM were blocked. Tests of the sensory neurons after nerve crush revealed profound hyperexcitability and synaptic facilitation, lasting weeks, that were specific to cells with axons in the crushed nerves. The long latency of the effects (1–2 days) suggested that signals from axonal damage were conveyed back to the soma and synapses by axonal transport. Of particular interest was the qualitative identity of the set of changes produced by axon crush with the alterations produced by neuromodulation and ADEM. One interpretation of these results is that a common set of mechanisms underlying long-term hyperexcitability and synaptic facilitation can be triggered by two signalling pathways from the periphery to the soma. The more primitive pathway would be provided by intracellular signals conveyed slowly by axonal transport from damaged axons. ADEM (conjoint electrical activation and extrinsic neuromodulation) of the soma during injury would provide a much more rapid signal of peripheral injury in nociceptors activated by injurious stimuli (see Billy and Walters, 1989a). ADEM may have evolved later, when the increased size of animals and resulting distances between nociceptor somata and their receptive processes selected for faster signals, so that regulation of protein synthesis necessary for injury-induced plasticity would not be delayed.

Although highly speculative, this hypothesis and our preliminary indications of links between cellular injury responses and long-term sensitization in *Aplysia* raise the possibility that some of the earliest forms of memory may have evolved from cellular repair and signal compensation mechanisms in primitive mechanosensory neurons. If, as brains became increasingly complex, these cells served as evolutionary precursors of other neuronal types, then primitive mechanisms of nociceptive plasticity might have been important for the evolution of other forms of memory as well. Indeed, both axon damage (*e.g.*, Janig, 1988; Kelly *et al.*, 1988) and learning (*e.g.*, Disterhoft *et al.*, 1986) have been associated with long-term hyperexcitability in mammalian neurons.

Evolutionary arguments have obvious weaknesses, especially when they deal with eras that left almost no fossil record. Nevertheless, such arguments can lead to fresh perspectives and novel physiological and molecular predictions. The present hypothesis makes three testable predictions: (1) that common cellular mechanisms (involving homologous molecules) contribute to nociceptive sensitization in a broad range of species; (2) that critical molecular steps are shared with mechanisms involved in cellular repair and signal compensation following axonal damage; and (3) that some of these molecular steps are shared with mechanisms involved in traditional forms of learning and memory. Systematic comparison of learning-related mechanisms and injury-related mechanisms in diverse animals will put these predictions to the test, and may provide insight into the evolution of some forms of memory.

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